



VERSION WITH MARKINGS TO SHOW CHANGES MADE

readily discerned from the following detailed description of exemplary embodiments thereof especially when read in conjunction with the drawings attached hereto.

Brief Description of the Drawings

5 In the drawings:

Figure 1 shows the chemical structures of dolastatin 10 (1a) and derivatives (1b-1e).

10 Figure 2 shows the *Cryptococcus neoformans* killing kinetics of dolastatin 10 and selected modifications where "killing" is shown as squares (no drug), triangles (1x MIC), inverted triangles (4x MIC), and diamonds (8x [the] MIC) for each compound.

Detailed Description of the Invention

Materials and Methods.

Antifungal agents.

15 Dolastatin 10 1a and modification 1d (Figure 1) were synthesized as described elsewhere (Pettit et al., 1989, *supra*; Pettit et al., 1996, *supra*; Pettit et al., Antineoplastic agents 365. Dolastatin 10 SAR probes, *Anti-Cancer Drug Design* 1997: In press). Synthesis of modification 1e is described in U.S. patent 5,663,149 (issued September 2, 1997), and synthesis of modifications 1b and 1c
20 are described in pending US applications SN *_____. The compounds were reconstituted in sterile dimethylsulfoxide (DMSO) immediately prior to all assays. DMSO alone had no detectable inhibitory effect on any of the tested microbes.

Fungal strains.

25 Clinical isolates of *C. neoformans* were obtained from patient cerebrospinal fluid, blood, bone marrow, sputum, bronchial lavage and wound infections at the University of Virginia Medical Center. Strains clinically resistant to fluconazole (See: Jessup, C.J. et al, 1997; Poster #F-88, 37th ICAAC, Toronto, Canada) were provided by the Center for Medical Mycology, Case
30 Western Reserve University. Yeast strains (except for *C. albidus* and *C. laurentii*) were maintained by single colony transfer on Sabouraud Dextrose Agar

Broth macrodilution assays were performed with RPMI prepared at pH 5, pH 6 and pH 7, and in RPMI, with and without 50% normal human serum (Lampire Biological Labs). *Cryptococcus neoformans* #90112 was used in each case.

Killing kinetics.

- 5 Overnight cultures of *C. neoformans* (#90112) in Ph 7.0 MOPS-buffered RPMI 1640 medium were inoculated into the same medium containing [1x the multiples of the broth macrodilution [MFC] MIC of the antifungal peptides, or an equivalent volume of DMSO. Cultures were shaken at 35°C, and aliquots aseptically removed at various times for dilution plating.

10 **Results and Discussion.**

- The initial screen for antimicrobial activity, the disk diffusion assay, suggested that dolastatin 10 and four analogs had narrow-spectrum antifungal activity (Table 1). Furthermore, at 100 µg/disk there was no inhibition of the tested bacterial strains (*see*, Materials and Methods, *supra*). The specificity for *C.*
- 15 *neoformans* was confirmed by broth macrodilution (Table 2). As with the disk diffusion technique, the parent compound was not growth inhibitory to the related species *C. albicans* and *C. laurentii*. *C. uniguttulatus* and *C. ater*. The MFCs for *C. neoformans* were typically identical or twofold greater than MICs. Exceptions occurred with *C. neoformans* #14116, where MFCs with compounds 1b and 1c were
- 20 sixteenfold greater than MICs. Dolastatin 10 was also fungicidal for strains of *C. neoformans* that were clinically resistant to fluconazole (Jessup et al., *supra*) (Table 3). As the methyl ester 1d was the most potent antifungal peptide in broth macrodilution tests, it was tested against 19 clinical isolates (did not include fluconazole-resistant strains) of *C. neoformans*. No resistant clinical isolates were
- 25 found.

Table 1. Antifungal activity of dolastatin 10 (1a) and modifications (1b-1e) in the disk diffusion assay.

| 5 | Organism | ATCC# | <u>1a</u> | <u>1b</u> | <u>1c</u> |
|-------|--------------------------------|-------|----------------|----------------|----------------|
| | | | MIC µg/disk | MIC µg/disk | MIC µg/disk |
| | <i>Cryptococcus neoformans</i> | 90112 | 25-50 | 3.12-6.25 | 1.56-3.12 |
| | <i>Cryptococcus albidus</i> | 66030 | >100 | >100 | [>100] |
| 10 | <i>Cryptococcus laurentii</i> | 66036 | >100 | >100 | [>100] |
| | <i>Candida albicans</i> | 90028 | >100 | >100 | >100 |
| | <i>Candida glabrata</i> | 90030 | >100 | >100 | >100 |
| <hr/> | | | | | |
| 15 | Organism | ATCC# | <u>1d</u> | <u>1e</u> | |
| | | | MIC µg/disk | MIC µg/disk | |
| | <i>Cryptococcus neoformans</i> | 90112 | 3.12-6.25 | 25-50 | |
| 20 | <i>Cryptococcus albidus</i> | 66030 | >100 | | |
| | <i>Cryptococcus laurentii</i> | 66036 | >100 | | |
| | <i>Candida albicans</i> | 90028 | >100 | >100 | |
| | <i>Candida glabrata</i> | 90030 | | | |

Table 2. Antifungal activity of dolastatin 10 (1a) and modifications (1b-1e) in the broth macrodilution assay.

| 30 | Organism | ATCC# | <u>1a</u> | | <u>1b</u> | | <u>1c</u> | |
|-------|--|-------|-----------|-------|-----------|-------|-----------|-------|
| | | | MIC | MFC | MIC | MFC | MIC | MFC |
| | | | µg/ml | µg/ml | µg/ml | µg/ml | µg/ml | µg/ml |
| <hr/> | | | | | | | | |
| | <i>Cryptococcus neoformans</i> | 66031 | 0.78 | 1.56 | 0.78 | 0.78 | 0.78 | 0.78 |
| | <i>Cryptococcus neoformans</i> | 14116 | 3.12 | 6.25 | 1.56 | 25 | 0.78 | 12.5 |
| 35 | <i>Cryptococcus neoformans</i> | 32045 | 0.78 | 1.56 | 0.78 | 0.78 | 0.78 | 1.56 |
| | <i>Cryptococcus neoformans</i> | 90112 | 0.78 | 1.56 | 1.56 | 3.12 | 0.78 | 0.78 |
| | <i>Cryptococcus albidus</i> | 66030 | >50 | | | | | |
| | <i>Cryptococcus albidus</i> ^a | 34140 | >50 | | | | | |
| | <i>Cryptococcus albidus</i> | 10666 | >50 | | | | | |
| 40 | <i>Cryptococcus laurentii</i> | 66036 | >50 | | | | | |
| | <i>Cryptococcus laurentii</i> | 18803 | >50 | | | | | |
| | <i>Cryptococcus laurentii</i> ^a | 34142 | >50 | | | | | |
| | <i>Cryptococcus uniguttulatus</i> ^a | 34143 | >50 | | | | | |
| | <i>Cryptococcus uniguttulatus</i> | 66033 | >50 | | | | | |

was no evidence of recovery. [The MFCs obtained after the recommended incubation period and one week later were compared. There was no evidence of recovery.]

5 The fungicidal action of four of the peptides was confirmed in killing kinetics experiments (Figure 2) (a paucity of modification 1c prohibited killing kinetics). In general, killing was concentration dependent between 1x and 4x the MIC, but not between 4x and 8x the MIC. The most dramatic reductions in CFUs were obtained with modification 1d.

10 Dolastatin 10 and three of the modifications were available in sufficient quantity to investigate the effects of two host factors, pH and serum, on broth macrodilution MICs and MFCs. The MICs and MFCs increased in acidified RPMI (Table 4). The anticytotoxic activity of modification 1d was the least affected by lowered pH. Attempts were made to obtain MICs at pH 8, but the strain did not grow in alkaline RPMI.

15 **Table 3. Inhibition of fluconazole-resistant^a *Cryptococcus neoformans* by dolastatin 10 (1a)**

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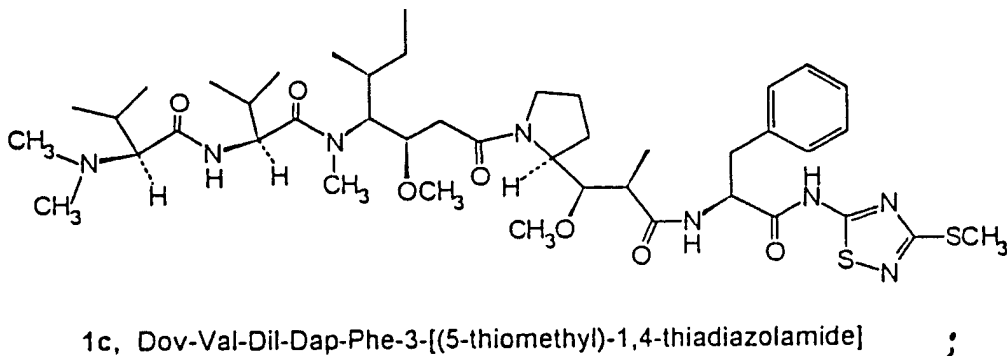
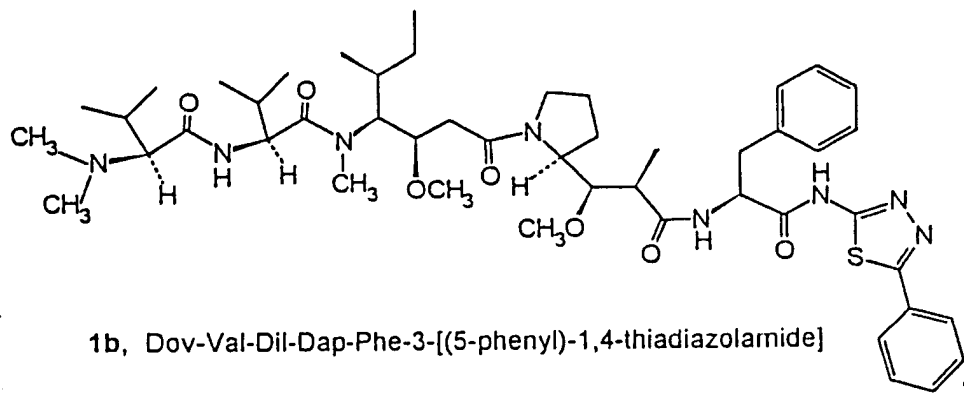
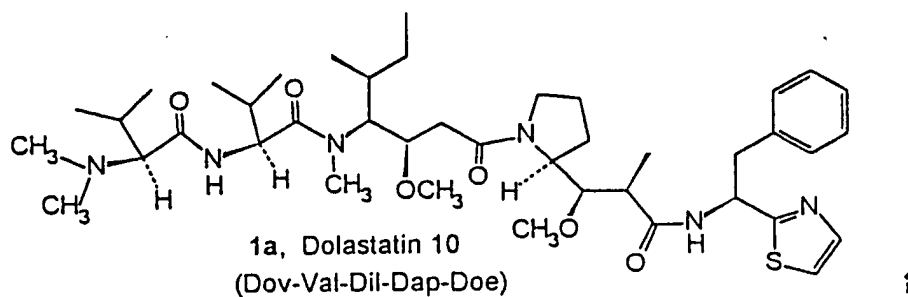
| Strain | MIC (µg/ml) | MFC (µg/ml) |
|------------|-------------|-------------|
| 94-2406 | 0.0487 | 0.0975 |
| 95-2792 | 0.78 | 3.12 |
| 25 96-2011 | 0.78 | 1.56 |
| 94-2483 | 0.195 | 0.39 |

^a Jessup, supra

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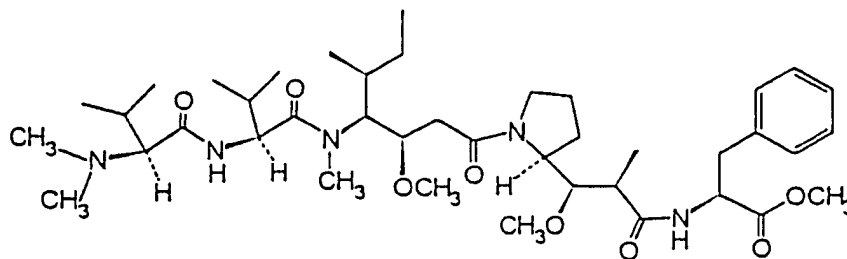
Claims

1. [A method of treating a host afflicted with a fungi-induced infection] A method of inhibiting fungal growth in a host comprising administering to said host [a pharmaceutically] an acceptable carrier and an effective amount of [an active ingredient] a compound selected from the group consisting of [the following chemical structures:] formulae 1a, 1b, 1c, 1d and 1e, wherein the structures of said formulae are as follows:



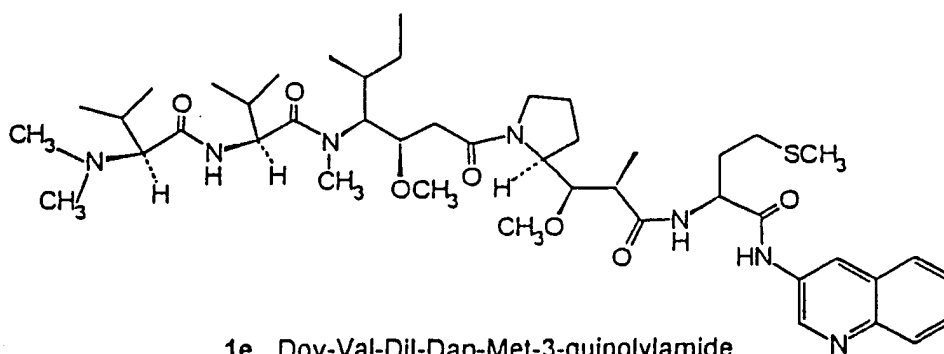
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1d, Dov-Val-Dil-Dap-Phe-OMe ;

10



1e, Dov-Val-Dil-Dap-Met-3-quinolylamide .

15

2. The method according to claim 1 in which said fungi [is] are *Cryptococcus neoformans*.
- 20 3. The method according to claim 1 in which said fungi induced infections are [Cryptococcus] cryptococcosis and epidermal and systemic infections resulting from contact with *Cryptococcus neoformans*.
4. The method according to claim [3] 9 in which said active ingredient is
- 25 administered to said host by parenteral means.
5. The method according to claim [3] 9 in which said active ingredient is administered topically to said host.

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6. The method according to claim [3] 9 in which said active ingredient is administered intravenously to said host.
- 5 7. The method according to claim [3] 9 in which said active ingredient is administered in a suppository inserted in said host.
8. The method according to claim 5 in which said [active ingredient is] [delivered in a carrier comprising a water and oil emulsion, petrolatum,]
10 carrier comprises a water-and-oil emulsion, petrolatum mineral oil, a moisturizer, a solubilizer and fragrance.
9. The method according to claim 3 wherein said host is a mammal.
- 15 10. The method according to claim 9 in which said mammal is a human.
11. The method according to claim 10 in which said active ingredient is administered to said host by parenteral means.
- 20 12. The method according to claim 10 in which said active ingredient is administered topically to said host.
13. The method according to claim 10 in which said active ingredient is administered intravenously to said host.
- 25 14. The method according to claim 10 in which said active ingredient is administered in a suppository inserted in said host.

15. The method according to claim 12 in which said carrier comprises a water-
and-oil emulsion, petrolatum, mineral oil, a moisturizer, a solubilizer and
fragrance.

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